preferably about sixfold, greater than the extent of microvascularization in the non-invasive, non-metastatic tumor sample.

In a more preferred embodiment, the determination of a poor prognosis is made when the level of nuclear localization of p53 in the tumor sample is from about twofold to about tenfold greater than the level of nuclear localization of p53 protein in the non-invasive, non-metastatic tumor sample, and the level of thrombospondin 1 expression in the tumor sample is from about twofold to about tenfold less than the level of thrombospondin 1 expression in the non-invasive, non-metastatic tumor sample and the extent of microvascularization in the tumor sample is from about twofold to about tenfold greater than the extent of microvascularization in the non-invasive, non-metastatic tumor sample. Most preferably, the level of nuclear localization of in the tumor sample is from about fivefold greater than the level of nuclear localization of p53 protein in the non-invasive, non-metastatic tumor sample, the level of thrombospondin 1 expression in the tumor sample is from about fivefold less than the level of thrombospondin 1 expression in the non-invasive, non-metastatic tumor sample and the extent of microvascularization in the tumor sample is from about sixfold greater than the extent of microvascularization in the non-invasive, non-metastatic tumor sample in determining a poor prognosis for a cancer patient.

In preferred embodiments, the levels of nuclear localization of p53, thrombospondin 1 expression and the extent of microvascularization are determined by immunohistochemical staining and detected by microscopy.

The invention also provides methods wherein the results of the determination of the levels of nuclear localization of p53, thrombospondin 1 expression, and the extent of microvascularization are used to prepare a prognostic or "risk" index for making a prognostic determination. In this aspect of the invention, a prognostic index is prepared comprising the product of the percentage of cells in the tumor sample that are positive for nuclear localization of p53 protein and one plus the intensity of immunohistochemical staining; the product of the percentage of cells in the tumor sample that are positive for microvascularization and one plus the intensity of immunohistochemical staining; and the product of the percentage of cells in the tumor sample that are positive for thrombospondin 1 expression and one plus the intensity of

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

immunohistochemical staining. In calculating these products, the intensity of staining is assigned a value of 0 for staining equal to a negative control, a value of 1 for weak staining greater than the negative control, a value of 2 for moderate staining intensity, a value of 3 for staining intensity equal to a positive control, and a value of 4 for staining intensity greater than the positive control. The calculated products of each of the tumor marker determinations are then weighted on a scale of from +1 to -4, and the index is produced as the sum of the weighted products for nuclear localization of p53, thrombospondin 1 expression and microvascularization. In the practice of the invention, a prognosis of a likelihood of further neoplastic, particularly metastatic, disease is made when this sum is less than about -5.

In additional embodiments, the prognostic index is produced by preparing a weighted scale of expression levels of the tumor markers related to progression observed in a representative sample of a particular tumor type, wherein the different values in the weighted scale are related to increased invasiveness or metastatic spread in the representative sample.

The methods of the invention are also provided for identifying a human cancer patient at risk for additional neoplastic disease, for staging malignant disease in a human cancer patient and assessing the relative risk of metastatic disease *versus* the risk of toxicity (such as leukocytopenia, *for example*) from chemotherapeutic treatment.

The methods of the invention are provided for prognosis of disease course in a cancer patient suffering from any specific cancer of any tissue of origin. In preferred embodiments, the cancer is breast cancer, prostate cancer or melanoma.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A through 1C are histograms of the intensity of tumor marker staining *versus* tumor histology for four histological subsets described in Example 1. The p values indicate significance of the observed differences between samples of different histologies, determined using paired, one-tail t-test analysis.

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

Figure 2 is a graph showing the increase in p53 nuclear accumulation and microvascularization and decrease in TSP-1 expression with tumor progression for a cohort of breast cancer samples as described in Example 1.

Figure 3 is a graph of a retrospective study of patient survival of 40 breast cancer patients as described in Example 2, comparing patients having a prognostic risk index of greater than or equal to -5 (GE -5) with patients having a prognostic risk index of less than -6 (LE -6).

Figure 4 is a graph of a retrospective study patient survival of 104 prostate cancer patients as described in Example 3, comparing patients having a prognostic risk index of greater than or equal to -7 (GE -7) with patients having a prognostic risk index of less than -8 (LE -8).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a method for making a prognosis about disease course in a human cancer patient. For the purposes of this invention, the term "prognosis" is intended to encompass predictions and likelihood analysis of disease progression, particularly tumor recurrence, metastatic spread and disease relapse. The prognostic methods of the invention are intended to be used clinically in making decisions concerning treatment modalities, including therapeutic intervention, diagnostic criteria such as disease staging, and disease monitoring and surveillance for metastasis or recurrence of neoplastic disease.

The methods of the invention are preferably performed using human cancer patient tumor samples, most preferably samples preserved, for example in paraffin, and prepared for histological and immunohistochemical analysis.

The invention also provides an index for use with the methods of the invention to relate three tumor markers (p53 nuclear accumulation, thrombospondin-1 expression and microvascularization) with disease progression, particularly invasiveness and metastatic spread. The indices of the invention can be prepared as described herein for any tumor type, provided that there is available a representative cohort of samples of the tumor type having varying degrees of tumor invasiveness and metastatic spread, to enable the production of a weighted scale of expression levels of the three tumor markers. Preferably, the size of the cohort is sufficiently

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001